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IDENTIFICATION OF GUT BACTERIA IN WORKER AND SOLDIER CASTES OF THE TERMITE *ANACANTHOTERMES AHNGERIANUS*

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Abstract: Cultivable gut bacteria from worker and soldier castes of *Anacanthotermes ahngerianus* were isolated and analysed using MALDI-TOF MS and Gram staining. Fifteen pure isolates were obtained: seven from workers and eight from soldiers. Worker isolates were assigned to *Enterobacter* (57.1%) and *Bacillus* (28.6%); one isolate (14.3%) remained unidentified. All soldier isolates were assigned to *Acinetobacter*. The results indicate differences in the taxonomic composition of cultivable gut bacteria between the two castes under the experimental conditions.

Keywords: *Anacanthotermes ahngerianus*; gut bacteria; worker caste; soldier caste; MALDI-TOF MS; Gram staining.

Introduction

Gut microorganisms play an important role in the digestion of cellulose-rich food by termites [1]. Studying the gut microbiota of *Anacanthotermes ahngerianus* is therefore relevant to understanding its feeding biology, identifying cellulose-degrading microorganisms and developing the biological basis of termite control. Termite guts harbour complex microbial communities involved in lignocellulose degradation. Because worker and soldier castes differ in colony functions and feeding characteristics, their cultivable gut bacterial communities may also differ. This study aimed to identify bacterial isolates from the guts of both castes using MALDI-TOF MS and to compare their taxonomic composition.

Materials and methods

Termite specimens were collected from Beruniy District, Republic of Karakalpakstan, in collaboration with the Republican Scientific and Production Center for Termite Control. Ten termites from each caste were used. Guts were dissected under aseptic conditions and homogenised in sterile phosphate buffer. The homogenates were diluted in sterile 0.9% NaCl solution and plated on meat-peptone agar. Cultures were incubated at 30 °C for 72 h. Morphologically distinct colonies were repeatedly subcultured to obtain pure cultures. Isolates were characterised using conventional methods, including Gram staining, and identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) [2].

Results

Fifteen pure bacterial isolates were obtained: seven from workers (WG-1, WG-2, WG-4, WG-6, WG-5/1, WG-5/2 and WG-5/3) and eight from soldiers (SG-1 to SG-8). Worker isolates WG-5/2 and WG-5/3 were Gram-positive; the remaining worker isolates and all soldier isolates

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were Gram-negative. These reactions were consistent with the general phenotypic characteristics of the bacterial taxa assigned by MALDI-TOF MS (Table 1).

Table 1. MALDI-TOF MS results for gut bacterial isolates from worker and soldier castes of *Anacanthotermes ahngerianus*

Caste	Isolate code	MALDI-TOF MS identification	Identification score
Worker	WG-5/1	Unidentified	1.47
Worker	WG-5/2	*Bacillus megaterium*	2.42
Worker	WG-5/3	*Bacillus subtilis*	2.04
Worker	WG-4	*Enterobacter cloacae*	2.05
Worker	WG-6	*Enterobacter cloacae*	2.26
Worker	WG-1	*Enterobacter asburiae*	1.95
Worker	WG-2	*Enterobacter cloacae*	2.21
Soldier	SG-1	*Acinetobacter junii*	1.98
Soldier	SG-2	*Acinetobacter parvus*	1.88
Soldier	SG-3	*Acinetobacter junii*	1.94
Soldier	SG-4	*Acinetobacter junii*	1.99
Soldier	SG-5	*Acinetobacter parvus*	2.10
Soldier	SG-6	*Acinetobacter pittii*	1.86
Soldier	SG-7	*Acinetobacter junii*	2.31
Soldier	SG-8	*Acinetobacter parvus*	2.04

Discussion

Among worker isolates, MALDI-TOF MS assignments comprised *Enterobacter* (57.1%) and *Bacillus* (28.6%), while one isolate (14.3%) remained unidentified. All eight soldier isolates were assigned to *Acinetobacter*. These findings indicate differences in the taxonomic composition of cultivable bacteria between termite castes under the experimental conditions.

Conclusion

A total of 15 pure bacterial isolates were recovered and analysed by MALDI-TOF MS. Worker isolates included representatives of *Bacillus* and *Enterobacter*, whereas all soldier isolates were assigned to *Acinetobacter*. Assessing their cellulolytic activity and confirming species-level identifications using molecular genetic methods are necessary to establish their functional roles in termite nutrition.

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